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Prevalence of colonisation with third-generation cephalosporin-resistant Enterobacteriaceae in ICU patients of Heidelberg University Hospitals

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ABSTRACT

The aim of this study was to assess colonisation and transmission of third-generation cephalosporin-resistant Enterobacteriaceae (CRE) from patients in 16 intensive care units. A prospective, repetitive point prevalence survey was performed over 6 months, involving samples from 1851 patients. CRE were isolated from 186 (10%) patients, with *Enterobacter* spp. being the most common. Mean point prevalence rates were significantly higher for paediatric wards (22.5%) compared to surgical (8.1%) and medical (5.5%) units. All CRE isolates were typed by pulsed-field gel electrophoresis. Non-outbreak nosocomial transmission rates of these pathogens were calculated as 12.8% for paediatric patients, compared to 6.8% for adult patients, which may reflect differences in sensitivity to overgrowth with resistant bacteria and contact with health care workers.

Keywords Third generation cephalosporin, resistance, Enterobacteriaceae, point prevalence survey, paediatric, PFGE

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INTRODUCTION

Isolation of third-generation cephalosporin-resistant Enterobacteriaceae (CRE) has been reported with increasing frequency for some years, often involving outbreaks in neonatal intensive care units (ICUs) [1–3]. Information about the prevalence of CRE in non-outbreak situations or other patient populations is limited, and is usually restricted to one or two specific ICUs [4,5]. The aim of this study was to investigate the prevalence of CRE colonisation in patients of all 16 ICUs of Heidelberg University Hospitals, and to assess the frequency of nosocomial transmission during ICU stay.

PATIENTS AND METHODS

Study design and setting

A prospective, repetitive point prevalence study was conducted over a 26-week period, starting in October 2000. Demographic and clinical data were obtained using a questionnaire. The severity of illness was assessed by the McCabe Jackson score [6]. The study was conducted at Heidelberg University Hospitals, a 1600-bed tertiary care facility with *c.* 60 000 admissions annually. All 16 ICUs participated, including three paediatric ICUs (neonatology (NICU), paediatric cardiology, general paediatrics), eight surgical ICUs (cardiac surgery, general surgery, transplantation surgery, urology, neurosurgery, orthopaedic surgery, gynaecology, thoracic surgery), one ICU with surgical (ear, nose and throat, and orthodontic surgery) and medical (neurological) patients, and four medical ICUs (cardio–pulmonary, gastrointestinal, stroke unit, haematology and bone marrow transplants). In these 16 ICUs, a minimum of six and a maximum of 16 beds were provided. Single bedrooms were available in 50% of the ICUs. Four of the ICUs did not treat mechanically ventilated patients.

Microbiological investigation

Peri-rectal swabs were taken weekly from all patients present on the ICU. All swabs were collected using a commercially available transport system (Trans-swab™; Mast, Reinfeld, Germany) and plated on sheep blood 5% agar and MacConkey agar.

For all aerobic Gram-negative organisms, susceptibility testing for cefpodoxime and ceftazidime was performed by agar disk diffusion tests on Mueller–Hinton agar. All isolates with a zone diameter of <22 mm for one of the antibiotics of interest (according to NCCLS criteria [7] for screening for enterobacteria producing extended-spectrum β-lactamases (ESBLs)) were identified to the species level by standard microbiological procedures (phenotypic characteristics, oxidase reaction) and a commercial microidentification system

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(API 20E and API 20:NE; bioMérieux, Marcy-l'Etoile, France). In addition, Etests (AB Biodisk, Solna, Sweden) were performed to determine the MICs of ceftazidime and cefpodoxime. Isolates resistant to ceftazidime (MIC ≥32 mg/L) and cefpodoxime (MIC ≥8 mg/L) were defined as CRE. The first isolate of each species/patient that was resistant to both third-generation cephalosporins was characterised genotypically by pulsed-field gel electrophoresis (PFGE) after digestion with the restriction enzyme *Spe*I [8].

Data analysis

The point prevalence rate was calculated for each unit on each day of investigation. The means of the weekly point prevalences were used to compare different units. An analysis of variance (ANOVA) was performed to investigate significant differences among the studied ICUs. Post-hoc analysis was performed by the Bonferroni method. All tests were performed two-tailed.

Two investigators compared the banding patterns resulting from PFGE independently. The criteria of Tenover *et al.* [9] were used for interpretation, with the modification that isolates were considered to be different strains if <90% of the bands matched (>3 bands different). If two patients carried a genotypically identical strain, this was considered as one transmission event. The transmission rate was calculated as the number of transmission events divided by 100 carriers of CRE.

RESULTS

During the study period, *c*. 5200 patients were admitted to the 16 ICUs. All patients present on one of the days of investigation were included in the study ($n = 1851$). Eighty-four (4.5%) study patients were admitted to more than one ICU during the study period. The study population was very heterogeneous and included neonates as well as nonagenarians. The proportion of patients with a McCabe–Jackson score of one, signifying a very poor prognosis, varied from 1% for the NICU to 45% for the neurological ICU. In the NICU and two medical ICUs, no patient had undergone a surgical procedure. The percentage of participants who had already received antibiotics when their first swab was taken varied from 15.9% to 81.8%, according to ICU. The antibiotics used most commonly were cephalosporins and penicillin + β-lactamase-inhibitor combinations (Table 1).

Peri-rectal swabs ($n = 3353$) were obtained from the study patients (mean = 1.8 swabs/patient). CRE were isolated from 328 (10%) swabs from 186 patients. *Enterobacter* spp. (71%), particularly *Enterobacter cloacae*, and *Citrobacter* spp. (20%) accounted for most resistant isolates. The species distribution of the isolates is presented in Table 2. Six patients carried eight ESBL-positive enterobacteria. The mean length of observation before the first CRE-positive swab was 23.3 days, compared to 12.9 days before discharge from the ICU for CRE-negative patients.

The weekly point prevalence rates varied considerably for each unit. Rates for three representative wards are presented in Fig. 1. The NICU had consistently high prevalence rates at the beginning of the investigation. An ongoing outbreak was suspected and interventional measures were initiated; this unit was therefore excluded from further analysis. The mean weekly point prevalence rates for the remaining 15 ICUs ranged from 1.3% on a medical ICU to 22.9% on a paediatric ICU (Fig. 2.). It was of interest that the prevalence rates in paediatric wards were significantly higher than in surgical or medical wards ($p < 0.001$). Although the prevalence rates were higher in surgical wards compared to medical wards, this difference did not reach statistical significance (p 0.087)

Isolates ($n = 189$) from 186 patients carrying CRE were typed by PFGE. Fourteen isolates (7.5%) were not viable or typable. Three patients carried more than one CRE. The suspected outbreak in the NICU was confirmed by the finding that 15 of 17 neonates carried an identical *E. cloacae* strain, accounting for a transmission rate of 88%. In the two paediatric ICUs, two PFGE clusters were delineated. Four paediatric patients were colonised with an identical *E. cloacae* strain, whereas six paediatric patients carried an identical *Enterobacter agglomerans* strain. In the 13 remaining non-paediatric ICUs, two patients each shared nine distinct *Enterobacter* strains. Thus, the transmission rate for children in a non-outbreak situation was 12.8%, compared to a transmission rate of 6.8% for adult patients.

In a hypothetical model, the prevalence rates for the two paediatric ICUs were calculated, excluding the patients for whom a nosocomial transmission was assumed. Nevertheless, the prevalence rates for these wards were higher (18.9 and 16.5) than for the non-paediatric wards. Of the colonised patients, 10% developed an infection with a CRE as the causative organism. Most of these patients had pneumonia, and septicaemia occurred in one patient.

Table 1. Basic characteristics of the participating ICUs and the study population

ICU	No. of beds	No. of respirators	No. of single rooms	No. of study patients	Mean age (range) of study patients	Rate of study patients with surgery	Rate of study patients with			Study patients 1st visit (%)	Predominant antibiotic therapy in study patients with AB	
							MC 1	MC 2	MC 3		1st choice	2nd choice
NICU	11	11	0	118	5 days (1–49 days)	0	1%	5%	94%			
P 2	12	12	2	90	2.8 years (1 day–17.8 years)	17.2%	10%	21%	69%	56.6	Ceph II + III	
P 1	12	9	0	86	2.5 years (1 day–18 years)	62.6%	3%	21%	76%	65.7	Ceph II + III	
S 2	12	12	0	144	63.4 years (4–95),	82.3%	25%	41%	34%	63.3	Ceph II + III	Metronidazole
S 4	6	6	4	74	58.5 years (17–92)	83.8%	30%	47%	23%	63.5	Ceph II + III	
S 5	10	4	2	109	58.8 years (6–92)	96.3%	13%	29%	58%	40.7	Ceph II	
S 1	16	16	0	164	68.8 years (7–87 years)	95.9%	2%	58%	40%	69.8	Quinolones	Ceph II + III
M 1	10	4	4	169	66.4 years (23–93)	4.6%	30%	44%	26%	51.4	Pen+β-lactam inhibitor	
M/S	10	10	2	109	62.4 years (31–90)	37.4%	45%	38%	17%	67	Pen+β-lactam inhibitor	
M 2	14	6	4	139	58.7 years (20–89)	7.7%	38%	40%	22%	81.8	Pen+β-lactam inhibitor	Macrolides
S 8	12	12	0	169	59.2 years (16–86)	76.9%	20%	57%	23%	33	Pen+β-lactam inhibitor	
S 7	8	0	1	80	47.6 years (24–78)	91.3%	6%	20%	74%	43.8	Ceph II	Metronidazole
S 6	12	12	0	102	56 years (12–81)	91.3%	28%	33%	39%	52.4	Ceph II + III	Pen+β-lactam inhibitor
M 3	16	0	0	251	62.2 years (19–90)	0	3%	31%	66%	15.9	Pen+β-lactam inhibitor	
S 3	8	0	0	88	57.1 years (17–87)	88.5%	5%	54%	41%	74	Ceph II + III	Co-trimoxazole
M 4	10	0	6	62	50.3 years (21–76)	0	19%	66%	15%	75.3	Co-trimoxazole	Quinolones

AB, antibiotics; Ceph, Cephalosporins; Pen, Penicillin, MC, McCabe–Jackson score; P, Paediatric ICU; S, Surgical ICU; M, Medical ICU.

Table 2. Species distribution of isolates resistant to third-generation cephalosporins

Species	% of CRE Isolates
Enterobacter cloacae	53
Citrobacter freundii	19.7
Enterobacter sakazakii	6.6
Enterobacter aerogenes	5.5
Enterobacter agglomerans	4.9
Morganella morganii	3.3
Escherichia coli	1.6
Hafnia alvei	1.6
Klebsiella pneumoniae	1.6
Citrobacter amalonaticus	0.55
Enterobacter amnigenus	0.55
Proteus vulgaris	0.55
Serratia liquefaciens	0.55

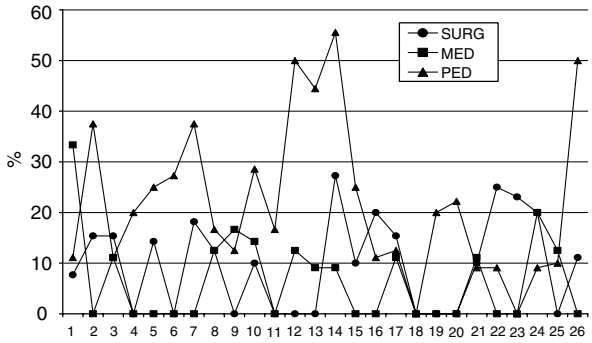

Fig. 1. Weekly point prevalence rates of colonisation with Enterobacteriaceae resistant to third-generation cephalosporins for three representative ICUs (paediatric, surgical and medical unit).

DISCUSSION

The occurrence of CRE has been reported for decades. Many authors have investigated outbreaks caused by ESBL-positive *Klebsiella* spp. or highly resistant *Enterobacter* spp. harbouring the *ampC* resistance gene [2,5,10,11]. However, data concerning the prevalence of CRE in a non-outbreak setting are limited. To our knowledge, this study is the first investigation describing the prevalence of CRE on 16 different ICUs simultaneously. Over a 26-week period, weekly point prevalence rates were consistently highest on the paediatric wards. Even when excluding children with an assumed nosocomial transmission of CRE from the calculation, the point prevalence rates on the paediatric wards remained higher than on the non-paediatric units.

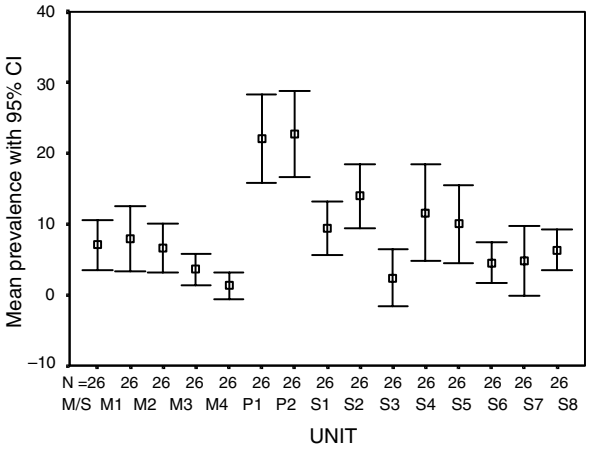

Fig. 2. Mean point prevalence rates with 95% confidence interval for 15 ICUs. Data for NICU not shown. P, paediatric ICU; S, surgical ICU; M, medical ICU.

Some theories on the mechanisms of nosocomial spread of CRE during a non-outbreak period have been formulated. Patients may acquire CRE endogenously or exogenously. Some authors have demonstrated that patients were colonised with susceptible Enterobacteriaceae that developed resistance whilst undergoing extensive antibiotic treatment [12,13]. Others observed that patients were already colonised with CRE on admission to the ICU. Antibiotic treatment favoured overgrowth of the resistant strains in the gastrointestinal tract, thereby selecting colonising strains from the patients' own gut flora [4,14,15]. The present study provided no data to differentiate between these two modes of colonisation. However, the sporadic transmission rates in the adult patient population support the theory that, at least in adult ICU patients, most CRE are acquired before admission to the ICU and that transmission is a rare event. Other authors have reported similar observations [15,16]. D'Agata *et al*. found a transmission rate of only 3% on surgical ICUs [4,17]. It can be concluded that horizontal transmission between adult ICU patients in a non-outbreak period is an unusual occurrence.

In contrast, children, and especially neonates, are prone to acquire CRE via patient-to-patient transmission. It has been reported that the gut of young children is especially sensitive to overgrowth with resistant bacteria [18–20]. A very young patient is therefore at higher risk of acquiring a multiresistant organism if these bacteria are present in sufficient frequency in the environment. It has been demonstrated that Enterobacteriaceae can survive in the inanimate environment [21] and can be recovered frequently from the hands of health care workers [2,3].

The impact of cephalosporin therapy on the development of cephalosporin-resistant Enterobacteriaceae has been discussed widely, although caution is required in drawing conclusions from ecological studies. It is worth noting that, in the present study, most patients in ICUs with weekly median point prevalence rates of >9% had received cephalosporins as a first-line therapy.

A two-fold preventative approach for limiting the spread of CRE in ICUs should be initiated, and can prove successful. De Man *et al*. [22] showed the positive impact of narrow-spectrum antibiotics as a first-line therapy in neonatal septicaemia. It was possible to reduce the proportion of resistant colonising Gram-negative flora by choosing antibiotics with less selective pressure on the mucosal environment [22]. Considering that the use of third-generation cephalosporins has been suggested as a risk factor for subsequent colonisation and/or infection with CRE, restricting the use of these substances for any environment with a high prevalence of these organisms should be discussed. The second approach involves the strict implementation of basic infection control practices, such as appropriate hand hygiene and control of environmental sources, especially in the care of neonates or young children.

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REFERENCES

1. Lucet JC, Decré D, Fichelle A *et al.* Control of a prolonged outbreak of extended-spectrum β-lactamase-producing enterobacteriaceae in a University Hospital. *Clin Infect Dis* 1999; **29**: 1411–1418.
2. van Nierop WH, Duse AG, Stewart RG, Bilgeri YR, Koornhof HJ. Molecular epidemiology of an outbreak of *Enterobacter cloacae* in the neonatal intensive care unit of a provincial hospital in Gauteng, South Africa. *J Clin Microbiol* 1998; **36**: 3085–3087.
3. Yu WL, Cheng HS, Lin HC, Peng CT, Tsai CH. Outbreak investigation of nosocomial *Enterobacter cloacae* bacteraemia in a neonatal intensive care unit. *Scand J Inf Dis* 2000; **32**: 293–298.

4. D'Agata E, Venkataraman L, DeGirolami PC *et al*. Colonization with broad-spectrum cephalosporin-resistant gram-negative bacilli in intensive care units during a nonoutbreak period: prevalence, risk factors, and rate of infection. *Crit Care Med* 1999; **27**: 1090–1095.
5. Toltzis P, Dul MJ, Hoyen C *et al*. Molecular epidemiology of antibiotic-resistant gram-negative bacilli in a neonatal intensive care unit during a nonoutbreak period. *Pediatrics* 2001; **108**: 1143–1148.
6. McCabe WR, Jackson GG. Gram-negative bacteremia. *Arch Intern Med* 1962; **110**: 847–855.
7. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility testing*, 7th edn. NCCLS Document M2-A7. Wayne, PA: NCCLS, 2000.
8. Pfaller MA, Hollis RJ, Sader HS. PFGE of chromosomal DNA., Section 10.5.c. In: H D Isenberg *et al*., eds. *Clinical microbiological procedures handbook.* Washington, DC: American Society for Microbiology, 1992.
9. Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.
10. Gonzales-Vertiz A, Alcantar-Curiel D, Cuauhtli M *et al*. Multiresistant extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* causing an outbreak of nosocomial bloodstream infection. *Infect Control Hosp Epidemiol* 2001; **22**: 723–725.
11. Grattard F, Pozzetto B, Berthelot P *et al*. Arbitrarily primed PCR, ribotyping, and plasmid pattern analysis applied to investigation of a nosocomial outbreak due to *Enterobacter cloacae* in a neonatal intensive care unit. *J Clin Microbiol* 1994; **32**: 596–602.
12. Chow JW, Fine MJ, Shlaes DM *et al*. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* 1991; **115**: 585–590.
13. Kaye KS, Cosgrove S, Harris A, Eliopoulos G, Carmeli Y. Risk factors for emergence of resistance to broad-spectrum cephalosporins among *Enterobacter* spp. *Antimicrob Agents Chemother* 2001; **45**: 2628–2630.
14. Toltzis P, Yamashita Vilt T, Blumer L, 1997 JL. Colonization with antibiotic-resistant gram-negative organisms in a pediatric intensive care unit. *Crit Care Med* 1997; **25**: 538–544.
15. Toltzis P, Hoyen C, Spinner-Block S, Salvator AE, Rice LB. Factors that predict preexisting colonization with antibiotic-resistant gram-negative bacilli in patients admitted to a pediatric intensive care unit. *Pediatrics* 1999; **103**: 719–723.
16. Chetchotisakd P, Phelps CL, Hartstein AI. Assessment of bacterial cross-transmission as a cause of infections in intensive care units. *Clin Infect Dis* 1994; **18**: 929–937.
17. D'Agata EMC, Venkataraman L, DeGirolami PC, Samore MH. Molecular epidemiology of acquisition of ceftazidime-resistant gram-negative bacilli in a nonoutbreak setting. *J Clin Microbiol* 1997; **35**: 2602–2605.
18. Almuneef MA, Baltimore RS, Farrel PA, Reagan-Cirincione P, Dembry LM. Molecular typing demonstrating transmission of gram-negative rods in a neonatal intensive care unit in the absence of a recognized epidemic. *Clin Infect Dis* 2001; **32**: 220–227.
19. Fryklund B, Tullus K, Berglund B, Burman LG. Importance of the environment and the faecal flora of infants, nursing staff and parents as sources of gram-negative bacteria colonizing newborns in three neonatal wards. *Infection* 1992; **20**: 253–257.
20. Goldmann DA. The bacterial flora of neonates in intensive care – monitoring and manipulation. *J Hosp Infect* 1988; **11** (suppl. A): 340–351.
21. D'Agata EMC, Venkataraman L, DeGirolami PC, Samore MH. Molecular epidemiology of ceftazidime-resistant gram-negative bacilli on inanimate surfaces and their role in cross-transmission during nonoutbreak periods. *J Clin Microbiol* 1999; **37**: 3065–3067.
22. De Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. *Lancet* 2000; **18**: 973–978.